are fundamental molecular motors that remodel RNA structures, DNA/RNA hybrids and RNA-protein complexes that are essential in gene regulation. So far their mechanism of action is very much debated and the classical assays are not sufficient to answer these questions this is particularly true for Dead box helicases. We have developed a cyclic assay in parallel on tens of molecules at the same time which allows detecting the unwinding of short RNA/DNA hybrids in real time by Ded1 helicase. The observation of unwinding by the Ded1 helicase in various conditions of ATP and analogs indicate that the enzyme melt the duplex rather than translocating along ssDNA and strip the complementary strand. On the contrary Upf1 helicase is a real translocase moving along nucleic acids over several hundred bases either on single strand or unwinding duplex.

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RecBCD Fails to Bypass the 5′-To-3′ Single-Stranded DNA Gap After Translocating Along Individual Chi-Containing Duplex DNA

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The E. coli RecBCD plays an important role of initiating the repair of double-stranded DNA breaks (DSB). Translocating and recognizing chi sequence (5'-GCTGGTGG-3') have been implicated with a conformational change that enables the enzyme to preserve the 3'-to-5' single-stranded DNA for RecA assembly. RecBCD is composed of three subunits, RecB (3'-to-5' helicase), RecD (5'-to-3' helicase), and RecC. Here we used a single-molecule tethered particle motion technique to directly monitor the translocation of RecBCD along chi-contained DNA molecules. Using bead-labeled enzymes, we monitored the RecBCD translocation along individual DNA by measuring the gradual decrease in the bead Brownian motion as the enzyme moves along the DNA towards the surface. DNA substrates were designed that RecBCD would encounter a single-stranded DNA gap after the recognition of the chi sequence. While translocating along chi-free DNA substrates, the time traces showed no apparent pause, which neither 3'-to-5' nor 5'-to-3' ssDNA gap influents the movement of the enzyme1. However, over 50% of RecBCD enzymes failed to pass through the 5'-to-3' ssDNA gap after translocating over chi-containing duplex DNA. Considering RecD as a major 5'-to-3' ssDNA translocase in the RecBCD complex, our observation is consistent with the model that the conformation change occurs after chi recognition and RecD is disengaged from the 5'-ssDNA.

(1) Chung, C.; Li, H. W. J. Am. Chem. Soc. 2013, 135, 8920.

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Single-Molecule Imaging Reveals the Translocation Dynamics of Hepatitis C Virus NS3 Helicase

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Worldwide, over 185 million people are chronically infected with hepatitis C virus (HCV), facing risks of developing liver diseases, such as hepatocarcinoma. There is no vaccine available. New therapies without side effects are highly needed. HCV encodes a superfamily 2 helicase (NS3h) in the C-terminal portion of nonstructural protein 3. This enzyme is essential for virus replication. Previous studies have well characterized the unwinding properties of the helicase. The ensemble approaches, however, have largely limited the understanding of the translocation dynamics. Here, we used optical traps to stretch kilobase-size single-stranded DNA (ssDNA), the single-molecule tracking of fluorescence-labeled NS3 enables us to directly determine the translocation speed, processivity, binding duration and the stoichiometry of translocating complex. Interestingly, we observed NS3h-mediated repetitive looping of ssDNA in the range of hundreds nucleotides. We further applied single-molecule fluorescence resonance energy transfer (smFRET) to the analysis of repetitive looping behavior. By tuning the fluorophore pair position between protein and nucleic acids, more structural information has been revealed. The dual-ways of movements observed by single molecule analysis may play roles in HCV life cycle.

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PcrA Helicase and the Mechanism of Asymmetric Rolling Circle DNA Replication

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DNA Helicases are responsible for the separation of double-stranded into single-stranded DNA for replication and other processes. Often working as part of a larger molecular machine, they are driven by ATP hydrolysis and translocate along the DNA, resulting in the separation of the DNA. One such helicase is PcrA, active during rolling circle replication of plasmids, carrying antibiotic-resistance genes and transferred between certain bacteria, thereby transmitting the resistance. To perform this function it forms part of a complex, in which it interacts with an initiator protein (in this case RepD) and a DNA polymerase III, which replicates one strand in the plasmid. Initiation occurs at the double stranded origin (in this case the oriD) when RepD nicks one strand. Following unwinding and replication of the unnicked strand, termination includes a series of strand exchanges resulting in one new plasmid and a circular single-strand that is replicated separately.

PcrA used in most previous structural and biochemical studies has been from Bacillus stearothermophilus, although the specific plasmids, polymerase and RepD are from Staphylococcus aureus. Because of potentially important protein-protein interactions, these will only be optimal with all components of the replication complex coming from the same organism. The effect of having all three proteins from S. aureus is now described. The rate of unwinding together with ATP usage is measured in this system. Protein-protein interactions and the details of termination have been little understood. Experiments are described to investigate what biochemical mechanism occurs, what are the main intermediates and how they interconvert.

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Analysis of Polymerase-DNA Interactions and Polymerase Activity with Electrically Actuated DNA Nanolevers on a Chip

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Next generation DNA sequencing technologies are on the verge of revolutionizing research and clinical genomics. An important cornerstone for ever faster, less expensive and more accurate genomic information are highly optimized and error-free DNA polymerases.

Here we present a novel method to study DNA polymerases and their interaction with nucleic acids on a chip. DNA molecules, which are end-tethered to microelectrodes on a chip, are set in motion by alternating electric fields, and the molecular dynamics of their oscillation (orientation switching) are measured by fluorescence energy transfer. The association and dissociation of various DNA polymerases are monitored in real-time by analyzing changes in the DNA motion that occur due to polymerase binding. Chemical rate constants of association and dissociation and affinity constants are determined.

For the polymerases phi29, Taq, and the Klenow fragment, the influences of temperature, mono- and divalent ion concentration, and presence/absence of dNTPs on polymerase binding kinetics are investigated. The polymerization activity is evaluated and even exonuclease activity can be observed in real-time. Simultaneously, information on the size of the DNA-polymerase complex is obtained with sub-nanometer accuracy and conformational changes in the ternary polymerase-dNTP-DNA complex are revealed (match/mismatch situation).

The method is label-free, uses a parallel microelectrode format for multiplexed assays and microfluidics for low sample consumption. It bears great potential as a powerful tool for the characterization of polymerases and facilitates the engineering of polymerases for, e.g., more efficient sequencing technologies.

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Studies of DNA Gyrase at the Single Molecule Level

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Topoisomerases are proteins that manipulate the topology of DNA. Unique among topoisomerases is DNA gyrase, a Type II bacterial topoisomerase, which is the only one capable of introducing negative supercoils. In addition, DNA gyrase can relax positive supercoils, an ability shared by other members of both Type I and II topoisomerases. To generate negative supercoils, DNA